REMARKS

In view of the amendments and remarks presented herein, the Applicants request withdrawal of the rejections and favorable reconsideration of the claims.

I. Status of the Claims

Claims 47-121 are pending in the instant application. Claims 102-121 have been withdrawn as directed to non-elected subject matter. Claims 47-101 stand variously rejected under 35 U.S.C. §102(b) and/or under 35 U.S.C. §103(a), and further under the judicially created doctrine of obviousness-type double patenting. Applicants respectfully traverse the rejections and request reconsideration in light of the above amendments and the following remarks.

II. Priority Claim

In the Office action, Applicants were reminded that if it is desired to claim priority under 35 U.S.C. §120 based upon a previously-filed application, specific reference to that earlier application must be made in the instant application. Applicants were further instructed that the specific reference must be submitted during the pendency of the application and within the later of four months from the actual filing date of the application or sixteen months from the filing date of the prior application.

Applicants do desire to claim priority to a prior application under 35 U.S.C. §120. However, Applicants have already amended the instant specification to make reference to the priority claim. That amendment to the specification was presented as a preliminary amendment on page 3 in the original filing papers (copy of that page is attached.)

Subsequent to the filing of the instant application, U.S. Serial No. 09/272,232 issued as U.S. Patent No. 6,401,267 on June 11, 2002. Applicants present the above amendment to update the status to said application in the current specification. This action

does not require a petition because the original claim to priority was timely presented at the time of filing.

III. Rejection under obviousness-type double patenting should be held in abeyance.

Claims 47-97, 99 and 101 were rejected under the doctrine of obviousness-type double patenting as being unpatentable over claims 1-36 of U.S. Patent No. 6,401,267. Claims 98 and 100 were rejected under the same doctrine over claims 1-40 of U.S. Patent No. 6,401,267 in view of Duck et al. (U.S. Patent No. 4,876,187).

Applicants respectfully request that the issue of obviousness-type double patenting be held in abeyance until such a time as Applicants have received an indication that the claims are otherwise in condition for allowance. At that time, Applicants will fully respond, should the rejections be maintained, by the submission of an appropriate terminal disclaimer. Applicants respectfully request the Examiner's discretion in this matter.

IV. Description of Sequencing by Hybridization.

Prior to elaborating on the distinctions between the present invention and the methods in the documents cited by the Examiner in the various art-based rejection, Applicants provide a discussion of the basic technology to which the present application is directed as this discussion will be helpful in clarifying the distinctions between the instant claims and the methods cited by the Examiner. The claims of the present invention are directed to methods of identifying a nucleotide sequence in a target nucleic acid using the technique of sequencing by hybridization (SBH). SBH determines the sequence of a target nucleic acid by using overlap of smaller oligomers that are constituents of the target nucleic acid (Strezoska *et al. Proc. Natl. Acad. Sci. USA*, 88:10089-10093, 1991; page 10089 second paragraph). Those of skill in that art, as early as 1992, recognized that fragments of up to 10kb can be ***sequenced*** using 50,000 to 100 000 probes using various algorithms (Drmanac *et al. Electrophoresis* 13:566-573, 1992, see abstract and second column of p566). In that paper, Drmanac *et al.* describe steps in the method (p568) including details of discriminative hybridization (e.g., see Figure 5); reading and quantifying the hybridization signals also is

discussed (see page 572). SBH 1, discussed in that paper involves taking an unknown target nucleic acid sequence and arraying (immobilizing) it on a solid support. These immobilized, unknown sequences are then interrogated using a labeled set of probes (See specification page 3, lines 1-15). The authors of Drmanac *et al.* go on to conclude that ***SBH 1*** will produce data on a megabase level (page 573). Southern *et al.* (Genomics, 13:1008-1017, 1992) recognize that it is possible to probe a complete base sequence with a complete set of oligonucleotides (p1008) and that sequence reconstruction and mutant detection can readily be accomplished using sequencing by hybridization (see Fig. 4 on page 1012).

In addition to SBH 1, those of skill in the art also were aware of a variation of the technique termed SBH 2, in which a sequencing chip is formed from an array of oligonucleotides of known sequence. In this format, the unknown target nucleic acids are labeled and allowed to hybridize to the immobilized oligonucleotides (See Southern 89/10977; Khrapko *et al.*, 1991).

Thus, the state of the art was such that, for example, an array of octomer probes could be produced which includes all the octomer probes that are perfectly complementary to a sample have a sequence of ATCAGGTCTGCATG (target sequence of 14 bases), then the following octomer probes will each form a completely matched hybrid with the sequence:

ATCAGGTC

TCAGGTC**T**

CAGGTCT**G**

AGGTCTG**C**

GGTCTGC**A**

GTCTGCA**T**

TCTGCAT**G**

Prior to the present invention, only SBH 1 and SBH 2 had been described. However, the present invention is directed to a new type of sequencing by hybridization

termed SBH format 3, which in contrast to the prior art methods uses hybridization with two sets of oligonucleotides of known sequence that can be used to interrogate the unknown target nucleic acid whose sequence is to be determined. In order to clarify that the claims of the present invention are directed to sequencing by hybridization, the preamble of claim 47 has been amended to recite: "A method of determining a nucleotide sequence of a target nucleic acid using sequencing by hybridization." This amendment is presented purely by way of clarification and is not necessitated to distinguish any of the art cited by the Examiner because the original claim 47 as filed encompassed and described the same steps of the technique as being claimed by the present, clarified claim language.

V. Rejection under 35 U.S.C. §102(b), should be withdrawn

Claims 47-55, 57, 59-75, 79-82, 84-91, 93-96 and 101 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Landegren et al. (U.S. Patent No. 4,988,617). Applicants respectfully traverse the rejection and request reconsideration in view of the present remarks.

The methods of the present invention are entirely distinguishable from the disclosure of Landegren *et al.*, because Landegren does not involve determining the sequence of a target nucleic acid, but is instead directed only to assessing whether or not a point mutation may be present in a given sequence. As discussed above, the technique of SBH in general involves compiling the actual nucleotide sequence of a given target nucleic acid, and not just detecting whether or not the target sequence contains a mismatch. The claims of the present invention are particularly directed to SBH format 3, an SBH technique that was described for the first time in the present application. In this technique, the sequence of a target nucleic acid is determined by:

> (a) contacting a target nucleic acid with a set of immobilized oligonucleotide probe(s) and at least one labeled oligonucleotide probe from a set of labeled oligonucleotide probes under hybridization conditions effective to permit hybridization between: (i) complementary sequences of the target nucleic acid and the immobilized probes and (ii) complementary sequences of the target nucleic acid and the labeled probe(s);

(b) covalently joining immobilized probe(s) and labeled probe(s) which are adjacently hybridized to the same target nucleic acid molecule;

(c) detecting the labels of the labeled oligonucleotide probe(s) that are covalently joined to the immobilized probe(s); and

(d) identifying at least one nucleotide sequence in the target nucleic acid by steps comprising connecting the nucleotide sequences of the detected labeled oligonucleotide probe(s) with the nucleotide sequences of their respective joined immobilized oligonucleotide probe(s).

Thus, the method described in claim 47 hybridizes the target nucleic acid (*i.e.*, the nucleic acid whose sequence is to be determined) with two sets of oligonucleotide probes (step a) in which the first set of probes is immobilized and the second set is labeled; step (b) involves covalently joining the labeled and immobilized probes that hybridize in positions adjacent to one another; step (c) involves detection of the covalently joined probes; and step (d) involves compiling the sequence of the target nucleic acid connecting the nucleotide sequences of the detected labeled oligonucleotide probe(s) with the nucleotide sequences of their respective joined immobilized oligonucleotide probe(s). This compiling step would provide the sequence of the target nucleic acid as discussed in Section IV above. This compiling step is nowhere disclosed in the Landegren document. In the absence of such a disclosure, Landegren cannot anticipate the claims of the present invention.

In contrast to the claims of the present invention, Landegren neither describes nor contemplates that the technique described therein could be used to determine the sequence the target nucleic acid. Rather, Landegren is directed to a "Method of Detecting a Change in Nucleic Acids," in which a target sequence is interrogated with the knowledge of the "known normal sequence" of that target and the knowledge of the "known possible mutation of at least one target nucleotide position" (Col. 2, lines 34-40). In performing this interrogation, the Landegren method requires that one of the two probes used (termed the "target probe" by Landegren) that "is complementary to and therefore capable of base pairing with either the normal or abnormal nucleotide at the corresponding target nucleotide position" (Col. 2 lines 48-50). The linking agent will only link the two probes "when the target nucleotide is correctly base paired . . . and if not correctly based paired the probes are

incapable of being covalently joined under such conditions." The presence or absence of the linking is used as the measure of the presence or absence of the ***known mutation*** in the interrogated nucleic acid. Thus, Landegren only is directed to detecting ***the presence or absence of a known*** point mutation in a target nucleic acid and does not provide a method of sequencing the target nucleic acid sequence.

Moreover, in reviewing the steps to be carried out in the Landegren method (see Col. 3, lines 1-21, and especially, line 17-18), it is a requirement in step (d) that the test substance (*i.e.*, the target nucleic acid whose sequence is being interrogated) is separated or melted away from the annealed probes. This step is not required in the methods of the present invention.

As the Landegren reference does not describe a method of sequencing a target nucleic acid, and further because the Landegren reference requires that in order to properly detect point mutations, the method must comprise a step in which the ligated probe products are separated from the target sequence before sequence analysis can be performed, Landegren does not anticipate claim 47 of the present application. Each of claims 48-55, 57, 59-75,79-82,84-91, 93-96 and 101, ultimately depend from claim 47, and therefore are novel over Landegren at least for the same reasons as claim 47 is novel. As such, Applicants respectfully request that the rejection of claims 47-55, 57, 59-75,79-82,84-91, 93-96 and 101 under 35 U.S.C. §102(b) based on Landegren be withdrawn and the claims be reconsidered for allowance.

VI. Rejection under 35 U.S.C. §103(a), should be withdrawn

Claims 56, 58, 76-78, 97 and 99 were rejected under 35 U.S.C. §103 as allegedly being obvious over Landegren in view of Cantor (U.S. Patent No. 5,503,980), claim 92 was rejected under 35 U.S.C. §103 over Landegren in view of Southern (WO 89/10977) and claims 98 and 100 were rejected under 35 U.S.C. §103 over Landegren in view of Duck (U.S. Patent No. 4,876,187). Applicants respectfully traverse these rejections.

In order to establish a *prima facie* case of obviousness the cited art must teach each element of the claimed invention. Moreover, where multiple references are used, there must be a suggestion or motivation to combine those references to arrive at the teachings of the invention and there must be a reasonable expectation of successfully realizing the invention in view of the teachings and the state of the art. *In re Vaeck*, 20 USPQ2d 1438, 1445 (Fed. Cir. 1991). These teachings must arise out of the prior art and not from the Applicants' own disclosure. As stated in MPEP 2143, these criteria must be met in order to properly establish *prima facie* obviousness. It is the Applicants' position that these criteria are not met by the teachings cited by the Examiner in the present case, and as such a *prima facie* case of obviousness has not been established.

For each of the rejections (*i.e.*, the rejection of claims 56, 58, 76-78, 97, and 99; claim 92; and claims 98 and 100) the Examiner relies on the disclosure of Landegren as a primary reference as generally teaching the methods of the invention claimed in the present application. However, as explained above, Applicants respectfully submit that the Examiner is incorrect in his assertions because the Landegren document does not teach a method of sequencing a target nucleic acid as claimed herein but rather is directed to a method of detecting the presence or absence of a point mutation. Moreover, the Landegren method requires that the sequence of the "normal sequence" and the "possible mutation" be known (Col. 2, lines 37-38). In addition, the Landegren method requires the step of separating the annealed ligated probes from the target nucleic acid. By contrast, the methods of the present invention require neither foreknowledge of the target sequence nor separation of the target sequence from the probes that are used to interrogate the target sequence.

With the above key to the differences and patentability of the instant claim over Landegren in mind, Applicants move on to discuss each of the combinations cited by the Examiner.

a. Each of claims 56, 58, 76-78, 97 and 99 is non-obvious over the Landegren/Cantor combination.

Adding the Cantor disclosure to the teachings of Landegren ***does not*** overcome the failings of Landegren. As discussed above, Landegren completely fails to suggest or motivate one of skill in the art to sequence a target nucleic acid using sequencing by hybridization. In fact, Landegren has nothing to do with sequencing and is merely directed to an overall method of detection where a known sequence is interrogated for the presence of a known mutation. Cantor is cited as teaching a sequencing chip, RNA probes with uracil and fragmentation of target nucleic acids with restriction enzymes. (Office action, page 7).

Nevertheless, the combination of Cantor and Landegren *et al.* does not teach or suggest a method of claim 47, which is directed to sequencing by hybridization in which two sets of probes, one immobilized, and the other labeled, are used compile the sequence of a target nucleic acid. In performing the SBH format 3 method claimed in claim 47, it is a requirement that the immobilized and labeled probes that hybridize to the target nucleic acid at positions immediately adjacent to each other are covalently linked to each other (see step (b) of claim 47). The limitations of claim 47 are incorporated into each of dependent claims 56, 58, 76-78, 97 and 99.

In order for Cantor to overcome the failings of Landegren, not only would Cantor have to teach sequencing chips, and/or uracil containing RNA probes and/or fragmentation of the target nucleic acids with restriction enzymes, it would also have to teach the basic method of SBH that Landegren has failed to teach. Applicants submit that Cantor ***does not disclose covalent joining of two probe molecules*** but instead teaches the covalent attachment of a hybridized target nucleic acid molecule to an immobilized oligonucleotide probe. Cantor, which employs one partially double-stranded probe that is attached to a solid support, and that may be ligated to the ***target nucleic acid*** upon hybridization with the target (see Cantor Example 3, column 13, lines 30-35). In addition, Cantor also fails to disclose two sets of probes (*i.e.*, a first set of probes attached to a solid support and the second set of

probes which are labeled probes in solution). Thus, much like Landegren, Cantor also fails to disclose the SBH 3 sequencing methods of the present invention.

Thus, regardless of whether or not Cantor provides a teaching of sequencing chips, and/or uracil containing RNA probes and/or fragmentation of the target nucleic acids with restriction enzymes, the basic combination of Landegren with Cantor remains flawed because it fails to teach SBH format 3 as described above. In the absence of such a teaching, the requirement that every element of the claimed method be taught by the cited art is not met, and therefore a *prima facie* case of obviousness cannot be established.

In light of the above comments, Applicants respectfully submit that claims 56, 58, 76-78, 97 and 99 are non-obvious over the combination of Landegren and Cantor and Applicants request that the rejection be withdrawn.

b. Claim 92 is non-obvious over the Landegren/Southern combination.

Claim 92 is directed to a method of SBH, in which the immobilized probes are immobilized on glass, polystyrene or Teflon. Regardless of the extent of Southern's teachings of immobilized probes on solid surfaces, Applicants submit that the addition of Southern to Landegren does not rehabilitate Landegren to provide a teaching of a method of sequencing by hybridization as claimed in the present application. As discussed briefly in Section IV above, the Southern document being cited by the Examiner describes SBH format 1. In that format, the unknown target nucleic acids are labeled and allowed to hybridize to the immobilized oligonucleotides. This is described in Southern at page 2, lines 3-13. Nowhere in Southern is there a technique described in which more than one set of probes (*i.e.*, a first set that is immobilized and a second set that is labeled) is used to determine the sequence of the target nucleic acid. And, as discussed above, Landegren is totally silent on actually determining the nucleotide sequence of the target nucleic acid using SBH, but is instead directed merely to detecting the presence or absence of a point mutation.

Thus, the combination of the two references as cited by the Examiner does not teach a sequencing by hybridization method in which (1) probes from a ***first*** **and** ***second*** set

of probes are hybridized to a nucleic acid molecule such that they are adjacent to each other and (2) a hybridized probe from a first set of oligonucleotide probes is covalently bonded to a hybridized probe from a second set of oligonucleotide probes. In the absence of these teachings, the elements of steps (a) and (b) of claim 47 are not met. Thus, regardless of the presence of the teachings in Southern of solid supports for use in SBH format 1, there is still no disclosure of the overall technique of SBH 3. This new and non-obvious method was the contribution of the instant inventors and was described for the first time in the priority application of the instant application.

In light of the above comments, Applicants respectfully submit that claim 92 is non-obvious over the cited art and that the rejections articulated by the Examiner at page 8 of the Office action should be withdrawn.

c. Claims 98 and 100 are non-obvious over the Landegren/Duck combination.

Claims 98 and 100 are respectively directed to the sequencing by hybridization methods of the invention in which the "covalently joined labeled probe comprising ribonucleotides is removed from the immobilized probe by RNAase treatment," (Claim 98) and "covalently joined labeled probe comprising a uracil base is removed from the immobilized probe by uracil-DNA glycosylase treatment." (Claim 100). The Examiner cites U.S. Patent No. 4,876,187 as teaching RNAse and uracil glycolase for washing unhybridized probes and indicated that such treatments may be combined with Landegren's detection methods. However, as Applicants have discussed above, the detection method of Landegren does not teach a method of sequencing the nucleic acid that is being detected. Duck does not rehabilitate Landegren, because Duck is merely directed to specific synthetic molecules that have a scissile bond that is cleaved upon hybridization in order to release the non-hybridized probe. This has nothing to do with sequencing of the target nucleic acid by hydridization with two separate sets of probes, covalently binding those probes that hybridize in positions adjacent to each other and subsequently compiling the sequence of the target nucleic acid. From the disclosure at column 3, lines 1-17 of the Duck patent, it would seem that the synthetic molecule of Duck is one which comprises a label and is designed to

hybridize to a nucleic acid molecule of interest. Once the synthetic molecule is hybridized to the molecule of interest, the hybridized complex is immobilized. Subsequently, the scissile bond is cleaved in such a manner as to leave the marker attached/hybridized to the nucleic acid molecule of interest. The presence of the marker on the immobilized nucleic acid molecule of interest facilitates the detection of the nucleic acid molecule. Thus, even if one of skill in the art were to combine both the Duck and Landegren references, the result of that combination would not be a method for sequencing by hybridization but would instead be a method of detecting a mutation in a nucleic acid (*i.e.*, the method of Landegren) in which method the specific synthetic molecules of Duck may be used to ***label*** the nucleic acid in which the mutation was being detected.

In light of the fact that neither Duck nor Landegren either alone or in combination teach any method of sequencing by hybridization, the mere fact that Duck teaches the use of RNAses and a uracil glycolase is insufficient to render obvious the main SBH methods of the invention, which necessarily are a part of each of claims 98 and 100. Applicants, therefore, respectfully submit that claims 98 and 100 are non-obvious over the cited art and that the rejections articulated by the Examiner at page 8-9 of the Office action should be withdrawn.

VII. Conclusions

Applicants believe that all of the rejections have been overcome and the claims of the instant application are now in condition for allowance and request an early indication of such a favorable disposition of the case. The Examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

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Respectfully submitted,

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